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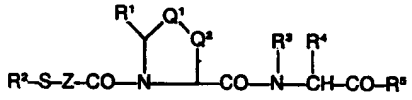
(71) Applicant: **SANTEN PHARMACEUTICAL CO., LTD., 9-19, 3-Chome, Shimoshinjo, Higashiyodogawa-ku Osaka533 (JP)**

(72) Inventor: **IWAO, Jun-ichi, 7-27, Nogami 4-chome Takarazuka-shi, Hyogo 665 (JP)**
Inventor: **OYA, Masayuki, 27-18, Yamatedai 3-chome Ibaraki-shi, Osaka 567 (JP)**
Inventor: **ISO, Tadashi, 197-7, Joroku, Sakai-shi, Osaka 588 (JP)**

(74) Representative: **Pearce, Anthony Richmond et al, Marks & Clerk Alpha Tower ATV Centre, Birmingham B1 1TT (GB)**

(54) SULFUR-CONTAINING ACYLAMINO ACIDS.

(57) Novel thiazolidine and pyrrolidine derivatives represented by the general formula:



(wherein Q^1 and Q^2 each represent a methylene group or a sulfur atom, Z represents an alkylene group containing 1 to 3 carbon atoms, R^1 represents a hydrogen atom, an alkyl group, an aromatic group or a heterocyclic group, R^2 represents a hydrogen atom, an alkyl group, an acyl group, an aromatic group, a heterocyclic group or a substituted mercapto group, R^3 represents a hydrogen atom or an alkyl group, R^4 represents a hydrogen atom, an alkyl group, an aromatic group, or R^3 and R^4 are taken together to form a pyrrolidine ring or a thiazolidine ring, and R^5 represents a hydroxy group or an amino group, with the respective groups being optionally substituted by an alkyl group or an aromatic group). These compounds are prepared by condensing a sulfur-containing acylthiazolidinecarboxylic acid or sulfur-containing acylpyrroildinecarboxylic acid with an amino acid. They are useful as antihypertensive agents.

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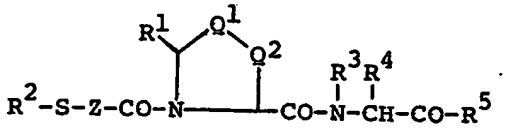
SPECIFICATION

Title of the Invention:

Sulfur-containing acylamino acids

This invention relates to sulfur-containing acylamino acids and related salts and antihypertensive compositions containing these compounds as main ingredients, which have the following formula



[I]

wherein

Q^1 and Q^2 are methylene or sulfur atom, but at least one of them is methylene;

Z is straight or branched alkylene which contains 1 to 3 carbon atoms;

R^1 is hydrogen, lower alkyl, cycloalkyl, higher alkyl, aralkyl, phenyl, furyl, thienyl, pyridyl or naphthyl, which may be substituted by 1 to 3 groups selected from lower alkyl, hydroxy, R^2-S-, lower alkoxy, halogen, nitro, amino, lower alkylamino, lower alkanoylamino, aroylamino, lower alkanoyloxy, aroyloxy, lower alkylenedioxy, carboxy, sulfamoyl, lower alkylaminosulfamoyl or cyano, but when R^1 is hydrogen, Q^1 and Q^2 are not methylene at the same time;

R^2 is hydrogen, lower alkyl, lower alkanoyl, cycloalkane-

carbonyl, higher alkanoyl, phenyl-lower alkanoyl, substituted phenyl-lower alkanoyl, benzoyl, substituted benzoyl, pyridylcarbonyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, or groups excluded hydrogen from R^6-S- or R^1;

R^3 is hydrogen or lower alkyl;

R^4 is hydrogen, lower alkyl, phenyl, aralkyl, pyrrolidine ring formed with R^3 or thiazolidine ring formed with R^3, which may be substituted by hydroxy, lower alkanoyloxy, aroyloxy, aralkyloxy, lower alkoxy, amino, guanidino, carboxy, lower alkoxycarbonyl, phenoxycarbonyl, aralkyloxycarbonyl, carbamoyl, mercapto, lower alkylthio, aralkylthio, lower alkanoylmercapto, aroylmercapto, imidazolyl or indolyl;

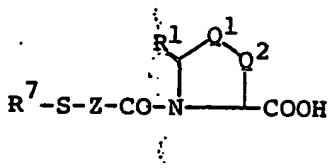
R^5 is hydroxy or amino, which may be substituted by lower alkyl, lower alkanoyloxy-lower alkyl, imido-lower alkyl, aralkyl or phenyl;

R^6 is lower alkenyl, higher alkenyl, tetrahydrofurfuryl or groups excluded hydrogen from R^1;

the same shall be applied hereinafter.

The compounds of this invention are synthesized by such methods as the following A, B and C.

A) The active derivatives of compounds represented by the formula



[II]

wherein

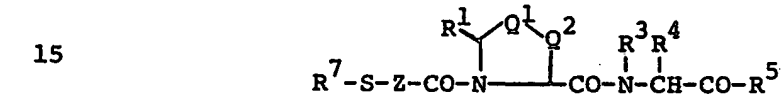
R^7 is groups excluded hydrogen from the above-mentioned R^2;

the same shall be applied hereinafter

and the compounds represented by the formula

[III]

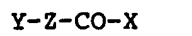
are condensed by a general method such as mixed anhydride method, etc. in synthesizing peptides to give the compounds of this invention represented by the formula.



[IV]

The resulting compounds are acidified with hydrochloric acid, trifluoroacetic acid, etc., alkalified with sodium hydroxide, ammonia, etc., or treated by catalytic hydrogenation with palladium-carbon, electrolytic reduction, or reduction with complex metal hydride such as sodium borohydride or with metal to give the compounds of this invention wherein R^2 is hydrogen and/or wherein R^5 is hydroxy. The diastereoisomers of the products can be separated and purified by a general method such as fractional recrystallization, chromatography, etc.

B) The compounds represented by the formula



[V]

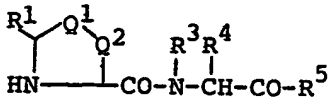
wherein

X is hydroxy or halogen;

Y is halogen;

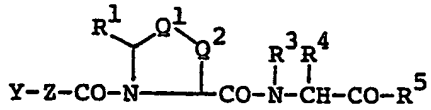
the same shall be applied hereinafter

react with the compounds represented by the formula



[VI]

by a general method such as Schotten-Baumann reaction, etc. to give the products represented by the formula.



[VII]

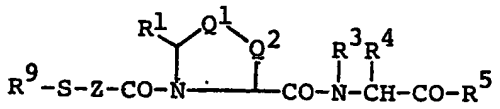
The products react with salt of benzylmercaptan, thioacetic acid or thiobenzoic acid such as potassium salt, etc. to give the compounds [IV] of this invention.

C) The compounds represented by the formula

$$R^8\text{-}S\text{-}Z\text{-}CO\text{-}X \qquad [VIII]$$

wherein

R^8 is lower alkyl, acyl such as acetyl, pivaloyl, benzoyl, etc., aralkyl such as benzyl, etc., X-CO-Z-S- or R^6-S- react with the above-mentioned compounds [VI] by the above method A or B to give the compounds of this invention represented by the formula



[IX]

wherein

R^9 is groups excluded hydrogen from the above mentioned R^2.

The compounds of this invention represented by the formula [I] synthesized by the above method A, B or C can form the conventional salts to be generally used as medicine such as sodium salt, potassium salt, calcium salt, aluminum salt, ammonium salt, diethylamine salt, triethanolamine salt, etc. The compounds [I] of this invention have the stereoisomers because they have one or more asymmetric carbon atoms. These stereoisomers are also within the limit of this invention. Examples are shown below, although this invention is not limited to these ones.

EXAMPLE 1

N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]glycine

To the solution of 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid dissolved in 30ml of dry tetrahydrofuran (It is abbreviated to THF hereinafter.) 0.51g of N-methylmorpholine is added. To the reaction mixture 0.68g of isobutyl chlorocarbonate is added at a temperature of -15 to -10°C. After stirring it for 30 minutes, the solution of 0.75g of glycine and 1.0g of

N-methylmorpholine dissolved in 5ml of THF and 20ml of water is added to it. After stirring it for 1 hour while being back to the room temperature gradually, and then removing THF in vacuo, the residue is acidified with N-HCl, and extracted with ethyl acetate. The organic layer is washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue is purified by silica gel column chromatography to give 1.44g (70%) of the titled compound.

mp 98-99°C (ethyl acetate-n-hexane)

$[\alpha]_D^{25}$ +126.4° (c=1.1, methanol)

IR (nujol, cm^{-1}, to be applied hereinafter unless specified)
3340, 1740, 1690, 1670, 1640, 1465, 1250, 1215, 775, 730

EXAMPLE 2

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]phenylalanine

To the solution of 3.55g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidine-carboxylic acid dissolved in 60ml of THF 1.0g of N-methyl-morpholine is added. To the reaction mixture 1.4g of iso-butyl chlorocarbonate is added at a temperature of -15 to -10°C. The suspension obtained by stirring it for 1 hour is added to 60ml of the aqueous solution of 3.38g of L-phenylalanine and 2.0g of triethylamine with stirring under ice-cooling. After stirring it under ice-cooling for 10 minutes and at room temperature for an additional 10 minutes, the solution is concentrated in vacuo. The concentrate is washed

with two 100-ml portions of ether, acidified with conc. hydrochloric acid, and extracted with ethyl acetate. The organic layer is washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated in vacuo to give 4.8g (96%) of the titled compound.

mp 91-95°C (amorphous powder)

$[\alpha]_D^{28}$ +151.2° (c=0.9, methanol)

IR 3280, 3130, 1728, 1680, 1655, 1625, 1600, 760

EXAMPLE 3

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]tryptophan

The suspension of mixed anhydride is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate in the same manner as Example 1. To the suspension 20ml of the aqueous solution of 2.04g of L-tryptophan and 1.0g of triethylamine are added. The mixture is stirred under ice-cooling for 30 minutes and at room temperature for an additional 30 minutes, and concentrated in vacuo. The concentrate is washed with ether, acidified with N-HCl, and filtered. The precipitate is washed well with dilute hydrochloric acid and water to give 1.7g (63%) of the titled compound.

mp 105-115°C (amorphous powder)

$[\alpha]_D^{28}$ +143.4° (c=0.5, methanol)

IR 3295, 1730, 1655, 1525, 1230, 1130, 960, 750

EXAMPLE 4

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]glutamic acid

The suspension of mixed anhydride in THF is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate. The suspension is added to the aqueous solution of 1.5g of L-glutamic acid and 2.0g of triethylamine, and treated in the same manner as Example 2 to give 1.9g (80%) of the titled compound.

mp 93-98°C (amorphous powder)

$[\alpha]_D^{28}$ +129.3° (c=1.0, methanol)

IR 3280, 1720, 1690, 1653, 1623, 1600, 762

EXAMPLE 5

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]serine

The suspension of mixed anhydride is prepared by using 3.55g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 1.0g of N-methylmorpholine, 1.4g of isobutyl chlorocarbonate and 60ml of THF in the same manner as Example 1. To the suspension 20ml of the aqueous solution of 2.1g of L-serine and 2.0g of triethylamine is added. The mixture is stirred under ice-cooling for 30 minutes and at room temperature for an additional 1 hour. After removing THF in vacuo, it is acidified with

N-HCl, and extracted with ethyl acetate. The organic layer is washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue is crystallized from benzene to give 3.6g (82%) of the titled compound.

mp 175.5-177.0°C (dec.) (ethanol-ether)

$[\alpha]_D^{32}$ +174.0° (c=0.5, methanol)

IR 3280, 1700, 1685, 1625, 1590, 1210. 755

EXAMPLE 6

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]leucine

The suspension of mixed anhydride in THF is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate. To the suspension the aqueous solution of 1.31g of L-leucine and 1.0g of triethylamine is added, and treated in the same manner as Example 1 to give 1.8g (77%) of the titled compound.

mp 78-84°C (amorphous powder)

$[\alpha]_D^{32}$ +115.2° (c=0.75, methanol)

IR 3220, 1720, 1650, 1620, 1230, 1130, 760

EXAMPLE 7

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]methionine

The suspension of mixed anhydride in THF is prepared by using 5.3g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 1.5g of N-methylmorpholine and 2.0g of isobutyl chlorocarbonate. To the suspension the aqueous solution of 4.5g of L-methionine and 3.0g of N-methylmorpholine is added, and treated in the same manner as Example 1 to give 1.6g (66%) of the titled compound.

mp 59-64°C (amorphous powder)

$[\alpha]_D^{30}$ +150.3° (c=0.8, methanol)

IR 3340, 1735, 1725, 1650, 1615, 1600, 755

EXAMPLE 8

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]tyrosine

The suspension of mixed anhydride in THF is prepared by using 3.55g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 1.0g of N-methylmorpholine and 1.4g of isobutyl chlorocarbonate. To the suspension 40ml of N-NaOH solution of 3.6g of L-tyrosine is added, and treated in the same manner as Example 1 to give 4.8g (93%) of the titled compound.

mp 95-96.5°C

$[\alpha]_D^{28}$ +141.8° (c=1.1, methanol)

IR 3240, 1730, 1690, 1660, 1630, 1225, 767, 726

EXAMPLE 9

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-

hydroxyphenyl)-4-thiazolidinyl]carbonyl]proline

The suspension of mixed anhydride in THF is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate. To the suspension the solution of I.0g of L-proline and 1.0g of triethylamine dissolved in aqueous THF is added, and treated in the same manner as Example 1 to give 1.5g (68%) of the titled compound.

mp 186-187°C (ethyl acetate)

$[\alpha]_D^{28}$ +122.3° (c=0.5, methanol)

IR 3295, 1750, 1685, 1635, 1600, 1235, 1170, 935, 760

EXAMPLE 10

N-[(2R,4R)-[3-(S-Benzoyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]glycine ethyl ester

To the solution of 2.09g of (2R,4R)-3-(S-benzoyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid dissolved in 30ml of dry THF, 0.51g of N-methylmorpholine is added. To the mixture 0.68g of isobutyl chlorocarbonate is added at a temperature of -15 to -10°C. After stirring it for 15 minutes, the solution of 0.7g of glycine ethyl ester hydrochloride and 0.51g of N-methylmorpholine dissolved in 5ml of THF and 15ml of water is added to it. After stirring it for 1 hour while being back to the room temperature gradually, and then removing THF in vacuo, the mixture is extracted with ethyl acetate. The organic layer is washed with N-HCl, water, saturated aqueous

sodium chloride solution, in order, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue is crystallized from ethyl acetate-benzene to give 2.43g (82%) of the titled compound.

mp 134-135°C (ethyl acetate)

$[\alpha]_D^{25}$ +95.4° (c=1.1, methanol)

IR 3270, 3060, 1749, 1690, 1655, 1630, 1456, 1203, 1042, 909, 788, 764, 723

EXAMPLE 11

N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]glycine ethyl ester

The suspension is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate. To the suspension the solution of 0.7g of glycine ethyl ester hydrochloride and 0.51g of N-methylmorpholine dissolved in aqueous THF is added, and treated in the same manner as Example 10 to give 1.8g(82%) of the titled compound.

mp 130-131°C (ethyl acetate-n-hexane)

$[\alpha]_D^{25}$ +119.0°C (c=0.9, methanol)

IR 3270, 3050, 1729, 1676, 1650 (shoulder), 1635, 1545, 1460, 1290, 1225, 760, 730

EXAMPLE 12

(2S)-N^2-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]histidine methyl ester

The suspension of mixed anhydride is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine, 0.68g of isobutyl chlorocarbonate and 30ml of dry THF in the same manner as Example 1. To the suspension the solution of 1.21g of L-histidine methyl ester dihydrochloride and 1.0g of triethylamine dissolved in 10ml of aqueous THF is added. After stirring it under ice-cooling for 30 minutes and at room temperature for an additional 30 minutes, and then removing THF in vacuo, the separated oil is obtained by decantation, and crystallized from aqueous sodium bicarbonate solution and benzene to give 1.4g (55%) of the titled compound.

mp 122-125°C (acetone-cyclohexane)

$[\alpha]_D^{28}$ +167.8° (c=0.5, methanol)

IR 3365, 3235, 1735, 1675, 1630, 1605, 1205, 1135, 945, 765

EXAMPLE 13

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]phenylalanine t-butyl ester

The suspension of mixed anhydride is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine, 0.68g of isobutyl chlorocarbonate and 30ml of dry THF in the same manner as Example 1. To the suspension the solution of 1.29g of L-phenylalanine t-butyl ester hydrochloride and 0.51g of N-methylmorpholine dissolved in

5ml of THF and 10ml of water is added. The mixture is stirred at room temperature for 1 hour, and then concentrated in vacuo. To the residue 50ml of water is added. The mixture is extracted with ethyl acetate. The or ic layer is washed with saturated aqueous sodium bicarbonate solution, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue is crystallized from n-hexane to give 2.02g (72%) of the titled compound.

mp 140-142°C (ethyl acetate-n-hexane)

$[\alpha]_D^{24}$ +126.1° (c=1.3, methanol)

IR 3360, 3260, 1700, 1680, 1625, 1580, 1295, 1271, 1245, 1235, 1222, 1150, 1026

EXAMPLE 14

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]alanine t-butyl ester

The suspension of mixed anhydride in THF is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid and 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate. To the suspension the solution of 0.9g of L-alanine t-butyl ester hydrochloride and 0.51g of N-methylmorpholine dissolved in dry THF is added, and treated in the same manner as Example 13. Thus obtained oil is purified by silica gel column chromatography to give 1.6g (66%) of the titled compound.

mp 170-171.5°C (dec.) (ethyl acetate-benzene)

$[\alpha]_D^{32}$ +98.7° (c=0.5, methanol)

IR 3360, 1700, 1685, 1595, 1450, 1130, 1050, 750

EXAMPLE 15

(2S)-N-[[1-(S-Benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinyl]carbonyl]phenylalanine t-butyl ester

To the solution of 1.43g of 1-(S-benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinecarboxylic acid (mp 89-92°C (dec.); $[\alpha]_D^{25}$ +47.4° (c=1.0, methanol)) and 0.3g of N-methylmorpholine dissolved in 30ml of dry THF 0.41g of isobutyl chlorocarbonate is added at a temperature of -15 to -10°C. After stirring it for 30 minutes, the solution of 0.77g of L-phenylalanine t-butyl ester hydrochloride and 0.3g of triethylamine dissolved in 10ml of aqueous THF is added to it. After stirring the mixture under ice-cooling for 30 minutes and at room temperature for an additional 30 minutes, and then removing THF in vacuo, the mixture is extracted with ethyl acetate. The organic layer is washed with saturated aqueous sodium bicarbonate solution, water and saturated aqueous sodium chloride solution, in order, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue is purified by silica gel column chromatography to give 1.5g (83%) of the titled compound.

$[\alpha]_D^{28}$ +20.5° (c=0.5, methanol)

IR (neat) 3275, 1730, 1660, 1625, 1600, 1525, 1205, 1155, 1035, 910, 845, 760

EXAMPLE 16

(2S)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]-o-t-butyltyrosine t-butyl ester

The suspension of mixed anhydride in THF is prepared by using 1.78g of (2R,4R)-3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate. To the suspension the solution of 1.47g of o-t-butyltyrosine t-butyl ester dissolved in dry THF is added, and the mixture is treated in the same manner as Example 13. The resulting residue is crystallized from ethyl acetate to give 2.65g (74%) of the titled compound.

mp 108-109°C (ethyl acetate)

$[\alpha]_D^{25}$ +97.3° (c=0.9, methanol)

IR 3320, 3260, 1740, 1725, 1699, 1663, 1630, 1440, 1384, 1265, 1220, 770, 735

EXAMPLE 17

(2S)-N-[(2R,4R)-[3-(S-Benzoyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]phenylalanine

To the suspension of 2.09g of (2R,4R)-3-(S-benzoyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid, 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate in THF, the solution of 1.65g of L-phenylalanine and 1.0g of triethylamine is added, and the mixture is treated in the same manner as Example 10. The resulting residue is purified by silica gel column chromatography to give 2.0g (71%) of the titled compound.

mp 95-100°C (amorphous powder)

$[\alpha]_D^{30}$ +131.3° (c=1.0, methanol)

IR 3260, 3120, 1730, 1720, 1650, 1630, 1600, 1200, 910, 755

EXAMPLE 18

(2S)-N-[(2R,4R)-[2-(2-Hydroxyphenyl)-3-(3-mercaptopropanoyl)-4-thiazolidinyl]carbonyl]phenylalanine

In 2ml of 28% ammonia water 0.5g of (2S)-N-[(2R,4R)-[3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]phenylalanine is dissolved. The solution is stirred at room temperature for 1 hour, and acidified with hydrochloric acid. The separated crystals are collected by filtration to give 0.4g (87%) of the titled compound.

mp 95-101°C (amorphous powder)

$[\alpha]_D^{28}$ +150.4° (c=0.9, methanol)

IR 3280, 3120, 1722, 1658, 1620, 1600, 762

EXAMPLE 19

(2S)-N-[(2R,4R)-[2-(2-Hydroxyphenyl)-3-(3-mercaptopropanoyl)-4-thiazolidinyl]carbonyl]serine

In 10ml of 28% ammonia water 0.5g of (2S)-N-[(2R,4R)-[3-(S-acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]serine is dissolved. The solution is stirred at room temperature for 30 minutes, and acidified with hydrochloric acid after removing excess ammonia. The separated oil is extracted with ethyl acetate. The organic layer is washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and ethyl acetate is removed to give 0.4g (88%) of the titled compound.

mp 140.5-145°C (ethyl acetate-benzene)

$[\alpha]_D^{32}$ +151.4° (c=0.5, methanol)

IR 3320, 1685, 1620, 1510, 1230, 1060, 760

EXAMPLE 20

(2S)-N-[[1-(S-Benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinyl]carbonyl]phenylalanine

The mixture of 0.7g of (2S)-N-[[1-(S-benzoyl-3-mercaptopropanoyl)-5-(2-hydroxyphenyl)-2-pyrrolidinyl]carbonyl]-phenylalanine t-butyl ester obtained in Example 15, 2.7g of trifluoroacetic acid and 0.6g of anisole is stirred at room temperature for 4 hours. After removing trifluoroacetic acid and anisole from it, the reaction mixture is purified by silica gel column chromatography to give 0.5g (79%) of the titled compound.

mp 72-102°C (amorphous powder)

$[\alpha]_D^{28}$ +41.7° (c=0.5, methanol)

IR 3295, 1735, 1655, 1600, 1525, 1205, 1040, 910, 760

EXAMPLE 21

(2R,2'R,4R,4'R)-3,3'-[3,3'-Dithiobis(propanoyl)]bis[4-[[(1S)-1-carboxy-2-hydroxy]ethylcarbamoyl]-2-(2-hydroxyphenyl)-thiazolidine]

(i) To the solution of 1.56g of (2R,2'R, 4R, 4'R)-3,3'-[3,3'-dithiobis(propanoyl)]bis[2-(2-hydroxyphenyl)-4-thiazolidinecarboxylic acid] and 0.51g of N-methylmorpholine dissolved in 30ml of dry THF, 0.68g of isobutyl chlorocarbonate is added at the temperature of -15 to -10°C. The mixture

is stirred for 1 hour, and the solution of 1.05g of L-serine and 1.0g of triethylamine dissolved in 10ml of water is added to it. The mixture is stirred under ice-cooling for 30 minutes and at room temperature for an additional 1 hour. After removing THF in vacuo, it is acidified with N-HCl, and extracted with ethyl acetate. The organic layer is washed with saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated in vacuo to give 1.4g (70%) of the titled compound.

mp 128.5-134.0°C (dec.)

$[\alpha]_D^{32}$ +116.6° (c=0.5, methanol)

IR 3280, 1730, 1665, 1630, 1460, 1240

(ii) To the solution of 0.2g of (2S)-N-[(2R,4R)-[2-(2-hydroxyphenyl)-3-(3-mercaptopropanoyl)-4-thiazolidinyl]-carbonyl]serine (TLC: Rf value[(a)] 0.67) dissolved in 5ml of methanol 5ml of 0.1N KI_3 is added dropwise. The mixture is stirred for 10 minutes, and then methanol is removed in vacuo. The separated crystals are collected by filtration to give 0.18g (90%) of the titled compound.

TLC: Rf value[(a)] 0.42

(a) Silica gel, chloroform-ethanol-acetic acid (5:5:1)

EXAMPLE 22

(2R,2'R,4R,4'R)-3,3'-[3,3'-Dithiobis(propanoyl)]bis[4-[[(1S)-1-carboxy-2-phenyl]ethylcarbamoyl]-2-(2-hydroxyphenyl)-thiazolidine]

(i) To the suspension of 1.56g of (2R,2'R,4R,4'R)-3,3'-[3,3'-dithiobis(propanoyl)]bis[2-(2-hydroxyphenyl)-4-

thiazolidinecarboxylic acid], 0.51g of N-methylmorpholine and 0.68g of isobutyl chlorocarbonate in THF the aqueous solution of 1.69g of L-phenylalanine and 1.0g of triethylamine is added. The mixture is treated in the same manner as Example 21 (i) to give 1.3g (72%) of the titled compound.
mp 136-139°C
$[\alpha]_D^{28}$ +139.4° (c=0.5, methanol)
IR 3290, 1728, 1660, 1625, 1600, 760

(ii) By substituting 0.46g of (2S)-N-[(2R,4R)-[2-(2-hydroxyphenyl)-3-(3-mercaptopropanoyl)-4-thiazolidinyl]-carbonyl]phenylalanine (TLC: Rf value(b) 0.44) in the procedure of Example 21 (ii), 0.43g (93%) of the titled compound is obtained.
TLC: Rf value(b) 0.28

(b) Silica gel, ethyl acetate-chloroform-acetic acid (10:5:3)

The following compounds are obtained in the same manner as the above examples.

(2R)-N-[(2R,4R)-[3-(S-Acetyl-3-mercaptopropanoyl)-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]-S-benzylcysteine

(2S)-N-[(2R,4R)-[3-[(2S)-S-Acetyl-3-mercapto-2-methyl-propanoyl]-2-(2-hydroxyphenyl)-4-thiazolidinyl]carbonyl]-phenylalanine

(2S)-N-[(2R,4R)-[3-(S-Pivaloyl-3-mercaptopropanoyl)-2-phenyl-4-thiazolidinyl]carbonyl]aspartic acid

(2S)-N^6-Acetyl-N^2-[(4R)-[3-(S-propanoyl-3-mercaptopropanoyl)-2-(4-methoxyphenyl)-4-thiazolidinyl]carbonyl]lysine

(2S)-N-[(4R)-[3-(S-(4-Methylbenzoyl)-3-mercaptopropanoyl]-2-(3,4-methylenedioxyphenyl)-4-thiazolidinyl]carbonyl]valine

(2S)-N-[(4R)-[3-[(2S)-S-Benzoyl-3-mercapto-2-methyl-propanoyl]-2-(4-pyridyl)-4-thiazolidinyl]carbonyl]phenyl-alanine

(2S)-N-[(4R)-[3-[(2S)-S-Benzoyl-3-mercapto-2-methyl-propanoyl]-2-(2-hydroxy-3-methoxyphenyl)-4-thiazolidinyl]-carbonyl]phenylalanine

(2R)-S-Acetyl-N-[(4R)-[3-[(2S)-S-acetyl-2-mercapto-propanoyl]-2-(2-thienyl)-4-thiazolidinyl]carbonyl]cysteine

(2S)-N-[(2R,4R)-[2-(2-hydroxyphenyl)-3-[3-[(tetrahydro-furfuryl)disulfanyl]propanoyl]-4-thiazolidinyl]carbonyl]-phenylalanine

(2S)-N-[(2R,4R)-[2-(2-Hydroxyphenyl)-3-[3-(aryl)disulfanyl]-propanoyl]-4-thiazolidinyl]carbonyl]phenylalanine

(2S)-N-[(4R)-[2-(4-Hydroxyphenyl)-3-[S-(3-pyridyl)mercapto-acetyl]-4-thiazolidinyl]carbonyl]isoleucine

It is clear that the compounds inhibiting angiotensin converting enzyme, which converts the biologically inactive decapeptide, angiotensin I to the active octapeptide, angiotensin II, may be antihypertensive drugs. Thus they were evaluated pharmacologically as an antihypertensive agent by measuring the inhibitory activity aganist the above enzyme.

PHARMACOLOGICAL TEST 1.

As the methods of measurement of angiotensin-converting enzyme activity, the bioassay for the contractile response of isolated smooth muscle or the pressor response of normal animals and the biochemical assay for the enzyme isolated from

lung or other organs of animals are known. The former is found more advantageous than the latter for the examination of the convertion of angiotensin I to angiotensin II in vivo. In this present study, therefore, we adopted the bioassay for contractile response of isolated guinea-pig ileum to angiotensin I.

Measurement of inhibitory activity of angiotensin-converting enzyme

Isolated guinea-pig ileum was prepared according to a general method. It was suspended in the organ bath containing 20ml of Tyrode's solution of 30°C gassed with 95% O_2 + 5% CO_2. The contraction induced by the addition of angiotensin I (final concentration 0.1μg/ml) at intervals of 10 minutes was recorded on a recticorder (Nihon Koden) for 90 seconds using FD pick up (ST-1T-H, Nihon Koden).

The test compounds were added to the both 5 minutes before the addition of angiotensin I.

The inhibitory activity of angiotensin-converting enzyme was calculated by the following formula.

$$\frac{A - B}{A} \times 100$$

A: the contractile intensity by angiotensin I before the addition of compound

B: the contractile intensity by angiotensin I after the addition of compound

From the fact that kininase II, which resolves bradykinin

contracting isolated guinea-pig ileum, is identical with angiotensin-converting enzyme, the augmentation of contractile response to bradykinin by test compounds was examined by using bradykinin (0.005μg/ml) in place of angiotensin I according to the above method. Consequently, the compounds of this invention obtained in Examples inhibited the contractile response to angiotensin I, and enhanced it to bradykinin.

PHAMRMACOLOGICAL TEST 2

The activity of angiotensin-converting enzyme was measured by spectrophotometry according to the method of Biochem. Pharmacol., 20, 1637 (1971). That is, the absorbance of hippuric acid was measured, which is liberated by incubating hippuryl-L-histidyl-L-leucine (HHL) as the substrate in the presence of angiotensin-converting enzyme extracted from rabbit lung.

Measurement of inhibitory activity of angiotensin-converting enzyme

The reaction mixture is as follows:

100mM phophate buffer (pH 8.3)

300mM sodium chloride

5mM HHL

10^{-3} to 10^{-9}M enzyme inhibitor

5mU enzyme

0.25ml of the above mixture was incubated at 37°C for 30 minutes, and the reaction was stopped by adding 0.25ml of 1N hydrochloric acid. To this solution 1.5ml of ethyl acetate

was added in order to extract hippuric acid. 1.0ml of ethyl acetate layer was evaporated to dryness, and the obtained residue was dissolved in 1.0ml of water. The absorbance of this solution was measured at 228nm.

The inhibitory activity of angiotensin-converting enzyme was calculated by the following formula.

$$\text{Percent inhibition} = \frac{A - B}{A} \times 100$$

A: the absorbance of reaction solution

B: the absorbance of reaction solution after the addition of compound

Concentration of compound producing 50% inhibition of angiotensin-converting enzyme (IC_{50})

The solution containing compound at the concentration of 1×10^{-3}M to 1×10^{-9}M was incubated, and the percent inhibition at each concentration was calculated according to the above formula. And then IC_{50}, the concentration of compound producing 50% inhibition of the enzyme activity, was determined. By the examination, the compounds of this invention were proved to inhibit angiotensin-converting enzyme as well as the known mercaptoacylamino acids.

PHARMACOLOGICAL TEST 3

Because recently it is clear that the compounds inhibiting angiotensin I-converting enzyme may be curative of not only renal hypertension but also essential hypertension, the compounds of this invention are estimated as an anti-

hypertensive agent by the following method.

Method

Male Wistar strain rats weighing 200-300g were used. Under ether anesthesia, polyethylene cannulae are inserted into carotid artery and jugular vein. The cannula to carotid artery is connected to an electric transducer, while the cannula to jugular vein is connected to an apparatus for continuous infusion. After the complete recovery from anesthesia, angiotensin I is infused intravenously in a dose of 300ng/kg by the apparatus for continuous infusion, and the pressor response is recorded by polygraph (Nihon Koden, RM-150). The compounds of this invention suspended in 0.5% tragacanth solution are administered orally in a dose of 0.3ml per 100g of body weight, and the pressor response to angiotensin I infused intravenously is measured with time.

Results

The compounds of this invention as well as the known antihypertensive mercaptoacylamino acids suppress the pressor response to angiotensin I by administering them orally to unanesthesized rats.

As exercised actually in using antihypertensive agents as the case may be, the compounds of this invention can be also given with the combination of diuretics. The compounds can be administered either orally or parenterally. The dosage forms are tablet, capsule, granule, powder, suppository, injection, etc. In the treatment of hypertension, these

preparations can contain not only general excipients but also other antihypertensive agents such as reserpine, α-methyldopa, guanethidine, clonidine, hydralazine, etc. The dose is adjusted depending on symptoms, dosage form, etc., but usual daily dosage is 1 to 5000mg, preferably 10 to 1000mg, in one or a few divided doses.

The followings show the examples of formulation.

(1) Oral drug

(a) tablets

compound of Example 2	30mg
lactose	150mg
crystalline cellulose	50mg
calcium carboxymethylcellulose	7mg
magnesium stearate	3mg
Total	240mg

compound of Example 5	150mg
lactose	60mg
crystalline cellulose	30mg
calcium carboxymethylcellulose	7mg
magnesium stearate	3mg
Total	250mg

The tablets may be treated with common film-coating and further with sugar-coating.

(b) granule

compound of Example 20	30mg

polyvinylpyrrolidone	25mg
lactose	385mg
hydroxypropylcellulose	50mg
talc	10mg
Total	500mg

(c) powder

compound of Example 9	30mg
lactose	500mg
starch	440mg
colloidal silica	30mg
Total	1000mg

compound of Example 8	300mg
lactose	230mg
starch	440mg
colloidal silica	30mg
Total	1000mg

(d) capsule

compound of Example 4	30mg
lactose	102mg
crystalline cellulose	56mg
colloical silica	2mg
Total	190mg

compound of Example 18	30mg
glycerin	349.98mg
butyl p-hydroxybenzoate	0.02mg

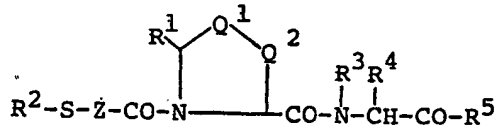
Total		380mg

(2) Injection

1 to 30mg of compound of Example 1 is contained in 1ml of the aqueous solution (pH 6.5-7.0).

What we claim is:

(1) Compounds and related salts which have the formula [I]



[I]

wherein

Q^1 and Q^2 are methylene or sulfur atom, but at least one of them is methylene;

Z is straight or branched alkylene which contains 1 to 3 carbon atoms;

R^1 is hydrogen, lower alkyl, cycloalkyl, higher alkyl, aralkyl, phenyl, furyl, thienyl, pyridyl or naphthyl, which may be substituted by 1 to 3 groups selected from lower alkyl, hydroxy, R^2-S-, lower alkoxy, halogen, nitro, amino, lower alkylamino, lower alkanoylamino, aroylamino, lower alkanoyloxy, aroyloxy, lower alkylenedioxy, carboxy, sulfamoyl, lower alkylaminosulfamoyl or cyano, but when R^1 is hydrogen, Q^1 and Q^2 are not methylene at the same time;

R^2 is hydrogen, lower alkyl, lower alkanoyl, cycloalkanecarbonyl, higher alkanoyl, phenyl-lower alkanoyl, substituted phenyl-lower alkanoyl, benzoyl, substituted benzoyl, pyridylcarbonyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, or groups excluded hydrogen from R^6-S- or R^1;

R^3 is hydrogen or lower alkyl;

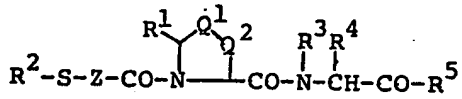
R^4 is hydrogen, lower alkyl, phenyl, aralkyl, pyrrolidine ring formed with R^3 or thiazolidine ring formed with R^3, which may be substituted by hydroxy, lower alkanoyloxy, aroyloxy, aralkyloxy, lower alkoxy, amino, guanidino, carboxy, lower alkoxycarbonyl, phenoxycarbonyl, aralkyloxycarbonyl, carbamoyl, mercapto, lower alkylthio, aralkylthio, lower alkanoylmercapto, aroylmercapto, imidazolyl or indolyl;

R^5 is hydroxy or amino, which may be substituted by lower alkyl, lower alkanoyloxy-lower alkyl, imido-lower alkyl, aralkyl or phenyl;

R^6 is lower alkenyl, higher alkenyl, tetrahydrofurfuryl or groups excluded hydrogen from R^1.

(2) Antihypertensive compositions containing compounds or related salts as main ingredients, which have the following formula [I]

[I]



wherein

Q^1 and Q^2 are methylene or sulfur atom, but at least one of them is methylene;

Z is straight or branched alkylene which contains 1 to 3 carbon atoms;

R^1 is hydrogen, lower alkyl, cycloalkyl, higher alkyl,

aralkyl, phenyl, furyl, thienyl, pyridyl or naphthyl, which may be substituted by 1 to 3 groups selected from lower alkyl, hydroxy, R^2-S-, lower alkoxy, halogen, nitro, amino, lower alkylamino, lower alkanoylamino, aroylamino, lower alkanoyloxy, aroyloxy, lower alkylenedioxy, carboxy, sulfamoyl, lower alkylaminosulfamoyl or cyano, but when R^1 is hydrogen, Q^1 and Q^2 are not methylene at the same time;

R^2 is hydrogen, lower alkyl, lower alkanoyl, cycloalkanecarbonyl, higher alkanoyl, phenyl-lower alkanoyl, substituted phenyl-lower alkanoyl, benzoyl, substituted benzoyl, pyridylcarbonyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, or groups excluded hydrogen from R^6-S- or R^1;

R^3 is hydrogen or lower alkyl;

R^4 is hydrogen, lower alkyl, phenyl, aralkyl, pyrrolidine ring formed with R^3 or thiazolidine ring formed with R^3, which may be substituted by hydroxy, lower alkanoyloxy, aroyloxy, aralkyloxy, lower alkoxy, amino, guanidino, carboxy, lower alkoxycarbonyl, phenoxycarbonyl, aralkyloxycarbonyl, carbamoyl, mercapto, lower alkylthio, aralkylthio, lower alkanoylmercapto, aroylmercapto, imidazolyl or indolyl;

R^5 is hydroxy or amino, which may be substituted by lower alkyl, lower alkanoyloxy-lower alkyl, imido-lower alkyl, aralkyl or phenyl;

R^6 is lower alkenyl, higher alkenyl, tetrahydrofurfuryl or groups excluded hydrogen from R^1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP81/00074

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[3] C07C 103/52, C07D 277/06, C07D 285/00, C07D 417/04, C07D 417/06, C07D 417/12, C07D 417/14, A61K 31/425, A61K 31/44, A61K 37/64

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
I P C	C07C 103/52, C07D 277/06, C07D 285/00, C07D 417/04, C07D 417/06, C07D 417/12, C07D 417/14, A61K 31/425, A61K 31/44, A61K 37/64

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	DE, A, 2,752,719, 1978-6-8 SQUIBB & SONS INC.	1 - 2
X	DE, A, 2,828,578, 1979-1-11 Yoshitomi Pharmaceutical Industries, Ltd.	1 - 2
X	DE, A, 2,842,100, 1979-4-5 SCIENCE UNION & CIE	1 - 2
X	DE, A, 2,854,877, 1979-6-28 SCIENCE UNION & CIE	1 - 2
X	DE, A, 2,914,059, 1979-10-25 Santen Seiyaku Kabushiki Kaisha, Yoshitomi Pharmaceutical Industries, Ltd.	1 - 2
X	EP, A, 0.001,978, 1979-5-30 Santen Seiyaku Kabushiki Kaisha,	1 - 2
X	JP, A, 54-100369, 1979-8-8 Santen Seiyaku Kabushiki Kaisha	1 - 2
X	JP, A, 54-154763, 1979-12-6 Yoshitomi Pharmaceutical Industries, Ltd.	1 - 2
X	JP, A, 55-9060, 1980-1-22 Yoshitomi Pharmaceutical Industries, Ltd.	1 - 2
X	JP, A, 55-11547, 1980-1-26 Santen Seiyaku Kabushiki Kaisha	1 - 2

[6] Special categories of cited documents: [15]

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
June 20, 1981 (20.06.81)	June 29, 1981 (29.06.81)
International Searching Authority [1]	**Signature of Authorized Officer [20]**
Japanese Patent Office	

International Application No. PCT/JP81/00074

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	JP, A, 55-22673, 1980-2-18 Yoshitomi Pharmaceutical Industries, Ltd.	1 - 2
X	Chemical Abstracts, Vol.88, No. 1, 1978-1-2, C. A. S. (Columbus Ohio U.S.A.) Ondetti Miguel A., et al., "Proline derivatives and converted compounds" See page 635, column 1, abstract No. 7376e Ger. Offen. 2,703,828	1 - 2
X	Chemical Abstracts, Vol. 91, No. 18, 1976-10-26, C. A. S. (Columbus Ohio U.S-A.) Horovitz Zola P., et al., "Phamaceutical composition containing a proline derivative and a diuretic" See page 331, column 2, abstract No. 145975k Ger. Offen. 2,854,316	1 - 2

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International Application No. PCT/JP81/00074

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Chemical Abstracts, Vol.92, No. 3, 1980-1-21, C. A. S. (Columbus Ohio U.S.A.) Wiskott Erik, "Amide of cyclic amino acids" See page 716, column 2, abstract No. 22816x Fr. Demande 2,407,204	1 - 2

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

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3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.